patient was able to urinate voluntarily, emptying the bladder, and the wound sinus was practically closed.

Remarks. Cases V and VI illustrate simple, straightforward suprapubic prostatectomies, presenting ideal results and the accomplishment of satisfactory drainage by means of irrigation and siphonage with a moderate sized tube; this permitted suture of the bladder walls around a small drainage opening, which, combined with the use of a retained catheter, was the means of affording a short and comfortable convalescence and early return to voluntary urination.

THE PRINCIPLES AND TECHNIQUE OF THE WASSERMAN AND NOGUCHI REACTIONS, AND THEIR COMPARATIVE VALUE TO THE CLINICIAN.

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Introduction. A syphilitic taint in an individual is of importance not only to the diagnostician and therapeutist, but also to the social economist. A means for its detection becomes a valuable contribution to our diagnostic methods, the more so the greater the sensitiveness of the reaction as established by its coincidence with clinical findings. The Wasserman reaction, based upon an ingenious principle, worked out by Bordet and Gengou, gives to the experienced laboratory worker a very satisfactory means of diagnosticating syphilitic affections, even in individuals who were infected years ago, of course excluding a dormant condition or patients at the end of a successful treatment. Having analyzed over 700 sera from dubious, negative, and distinctly luetic individuals, I venture to give my results with the Wasserman and Noguchi tests, considering as an introduction the various principles of immunity upon which the reaction of complement deviation depends.

Reaction of the Organism to Infection. When a child is affected with diphtheria, the poison produced by this bacterium produces most of the symptoms of the disease. When the patient survives, it is possible to demonstrate in his serum substances which are capable of neutralizing the toxin of the diphtheria bacillus. The antitoxin to this disease is extensively manufactured by drug concerns by infecting horses with diphtheria, and at a convenient time withdrawing from the jugular vein some serum which contains the antitoxin. We learn from this simple exposition that an organism infected with a certain disease, in order to protect itself, produces in its body a substance which neutralizes the bad effect of the invading factor. Not only does the animal body respond in such a specific manner against bacteria, but the same is evident when we inject certain acellular vegetable poisons or cells from another animal.

It is by no means a difficult task to produce in the serum of a suitable animal substances which will destroy the red blood corpuscles from another animal. This is accomplished by injecting into the animal such cells as we wish to destroy. One may produce in the rabbit (the animal used for the production of antibody) substances which are capable of destroying sheep red cells, bovine red cells, or human red cells, the specific quality depending upon the cells used for immunization.

In order to be able to grasp the steps in the Wasserman reaction it is indispensable to be acquainted with the steps involved in demonstrating antibodies of any kind formed accidentally (by disease) or purposely (in immunizing) in the body fluids of an animal. Ehrlich showed long ago that in the demonstration of antibodies, or, as he called them, amboceptors, three distinctly different substances are required in order to form a complete reaction: first, the cell or poison against which we wish to immunize, or, more plainly, against which we desire to obtain an antibody; second, the antibody (or amboceptor) obtained by repeated injections of the special cell or poison into the rabbit (or any other suitable animal); third, a completing substance—the complement. This latter substance is present in variable quantities in the sera of all animals, its quantity being rather constant in guinea-pigs. It is destroyed by heating serum at 56° or 57° for one-half hour, and is similarly affected by various other physical agents. This is not the case with antibodies which are comparatively thermostable. We have, then, three factors: (1) A cell to be destroyed or a poison to be neutralized; (2) a substance capable of doing this—the amboceptor or antibody; and (3) a completing substance, without which the reaction cannot take place—the complement.

Ehrlich and others, in order to impress the reaction upon the minds of men interested in immune processes, made use of diagrams. To make it still more familiar, let a lock represent the cell; a key which fits it the antibody, and the hand that will turn the key the complement. By giving a lock to a smith we can get a key made to fit the specific lock exactly. When we inject cells we get an antibody which exactly fits the cell injected, and the same is true when we inject a bacillus or a poison. All these substances capable of producing antibodies (antibody generators) are known as antigens.

To determine whether a bacterium was killed or a poison neutralized by being exposed to the action of a specific amboceptor is not as simple a process as the demonstration of the destruction of red blood corpuscles by an amboceptor directed against them. A suspension of red blood corpuscles minus amboceptor and complement gives an opaque red mixture; when we add the amboceptor plus complement and incubate at 37°, the opacity disappears and a clear red fluid results. It is apparent that hemolysis or destruction of red cells is a phenomenon that can readily be seen in vitro, and its presence signifies that the three substances spoken of above are present in the test-tube. If any one of the three is not there, or is present in an inactive state, the red cells will remain unaffected and the mixture will retain an opaque red color.

HEMOLYSIS. The phenomenon known as hemolysis depends upon the destruction of red blood corpuscles. There are many reagents capable of doing this, such as distilled water, acids, and alkalies. It is also possible to form in warm-blooded animals substances which will bring about hemolysis against certain red blood cells. This is accomplished by injecting an animal (a rabbit or goat) with the cells of a sheep or any other animal. The serum from such a rabbit, when brought in contact with the cells of the sheep, will cause the mixture to become clear (hemolysis). The same amount of serum from an untreated rabbit will have no effect on a similar suspension of sheep cells. The substance produced in the rabbit's serum is known as the antisheep amboceptor, and together with sheep cells and complement (from a guinea-pig) is known as a hemolytic system.

THE BORDET-GENGOU PHENOMENON. The principle applied in the Wasserman reaction depends upon a test originally devised by Bordet and Gengou in 1901. At that time these authors conceived the idea of demonstrating in vitro the presence of unknown amboceptors (or antibodies) by attempting to fit on to them the corresponding antigen. It is clear that in order to do this, complement is absolutely necessary. Supposing, then, the antibody at hand to be from a patient who suffered from typhoid, this antibody ought to anchor itself to the organism responsible for its specific production, the typhoid bacillus, or suitable extracts made from typhoid cultures. This actually takes place and, if complement is present, forms an undisruptible triad; in other words, typhoid antigen is bound to the available complement by the typhoid amboceptor. If we should introduce into the very same test-tube a mixture consisting of sheep cells and some antisheep amboceptor, no hemolysis will take place, for the simple reason that in order to do so complement is necessary, and, as said before, the available complement has been bound—or deviated—by the preëxisting well-fitting typhoid antigen and typhoid antibody. If the serum of the patient had not contained typhoid antibodies (in case of acute miliary tuberculosis), the complement would not have been bound to the typhoid antigen, but would have been left free, and a hemolytic system consisting of sheep cells and antisheep amboceptor would be completely laked—or dissolved—for the available complement was left free to act, was not deviated or bound up.

We learn from the above exposition that in order to prove the presence or absence of certain antibodies, we make use of the phenomenon of bound or unbound complement, utilizing a hemolytic system simply as an indicator. Exactly the same principle is applied to the serum diagnosis of syphilis. Unable to produce a growth of Spirochæta pallida upon any culture medium, we have to be contented with organ extracts containing them in greatest numbers; for this purpose the liver of the luetic fœtus furnishes the requirements. The extract produced is known as luetic antigen, and need not confuse anybody, for we know that an antigen is a body capable of forming antibodies. If an individual has had syphilis some years ago, he would also have syphilitic antibodies in his serum which, when brought in contact with the extract from the syphilitic liver, would invariably, as pointed out before, bind complement, and a hemolytic system (sheep cells or any other red cells with the corresponding amboceptors) will never be affected on account of lack of complement.

I hope the very rudimentary style of offering to the reader an explanation of the phenomenon of complement deviation will be remembered on account of the simplicity of terms used and the simple analogy with more familiar subjects. I will now consider the actual steps of the reaction, giving it in detail and as plainly as the untrained worker desires it.

TECHNIQUE OF THE WASSERMAN REACTION. *Principle Involved.* As mentioned before, antibodies will attract complement if the antigen responsible for their formation is present in the same test-tube. In the Wasserman reaction a serum containing antibodies capable of uniting with the antigen used (a substance containing lipoids), and thus deviate the introduced complement, will not permit hemolysis to occur if sheep cells and their antisheep amboceptor are subsequently placed in the same tube, and for obvious reasons—the complement was bound or deviated previously by conditions suitable for such an interaction. If the patient's serum does not contain the required antibody, the introduced complement will remain unbound and in a fit condition to destroy the sheep cells when subsequently introduced with their antisheep amboceptor.

Modus Operandi. (1) Obtaining blood from patient: A fairly stout piece of rubber tubing is placed a little above the elbow and held snugly in place by an artery clamp. Do not obliterate the pulse. This brings into prominence the veins at the bend of the elbow. To a stout hypodermic needle (I use a 19 bore—one and one-half inch needle) attach a two inch piece of rubber tubing. Holding the free end of the rubber tubing in an ordinary sterile test-tube, quickly plunge the needle into the most prominent vein; if expertly done, the patient will hardly feel it and the blood will immediately begin to flow. About 6 to 10 c.c. of blood is withdrawn and placed in the ice box over night to coagulate. The serum separates and may be pipetted off absolutely clear without cells. It is advisable to take the blood as far from a meal as possible, as proximity to a meal makes the blood lipemic, interfering with perfect working conditions. (2) Having obtained 1 or 2 c.c. of clear serum, it is placed in a test-tube in the thermostat at 56° for one hour. Care must be taken not to permit the heat to rise too high (over 58°). (3) After this, 0.2 c.c. is placed in each of two test-tubes, one the test, the other the control. (4) To each is now added 0.1 c.c. fresh complement. (5) To the test portion is added one unit of antigen. The control does not receive any antigen. (6) Each tube receives now 3 c.c. of a 0.95 per cent. NaCl solution. In order to be able to judge properly the correctness of the procedure, the more controls one has the better; it is therefore necessary to compare the serum to be tested with two sera from known positive and negative bloods. (7) Shake every tube well and place in incubator at 37° or 38° for one hour. During this time, if the serum is luetic the antibodies present will, together with the antigen, bind the complement and render it inactive for hemolysis. (8) After one hour incubation each tube receives two units of amboceptor and 1 c.c. of a 5 per cent. suspension of sheep cells in 0.95 NaCl. The tubes are again vigorously shaken and placed in the incubator at 37° and inspected after ten minutes. If the reagents are properly adjusted hemolysis begins in the control tubes in fifteen to twenty minutes, and careful watching becomes a very essential point at this stage of the test. As soon as the control is completely hemolyzed the tubes are to be compared; only those should be pronounced negative that show a transparent fluid the same as the control.

Permitting the tubes to stand undisturbed in a cool place (15° to 17°) for twenty-four hours shows in the positive test a deposit of red cells, the size of the deposit depending upon the severity of the infection or proximity to the initial lesion as well as upon the degree of balance of the reagents used. Usually a markedly positive serum gives at the end of twenty-four hours a clear supernatant fluid of a light pink hue with a bordeaux red accumulation on the bottom of the tube. The weaker the reaction, the redder the supernatant fluid and the scantier the deposit of cells. In testing more than one serum, the reaction in each individual test must be considered as finished as soon as the controls are completely hemolyzed, in which case the two tubes are immediately removed to a cool place.

TECHNIQUE OF THE NOGUCHI REACTION. *Principles Involved.* These are same as in the Wasserman, excepting that the amboceptor is directed against human cells, for reasons to be spoken of later. It also facilitates the handling of reagents, as they are mostly paper soaked in the antigen and amboceptor. These do not readily deteriorate, as is the case with fluid biological reagents. The serum does not need inactivation at 56°.

Modus Operandi. (1) With a capillary pipette allow one drop of fresh serum to fall into a narrow (1 c.m. lumen) test-tube. The pipette is not to be used for any other serum. (2) Add 0.05 c.c.

fresh complement. (3) To the front row (rear row for control) add one piece (more or less, depending on the titre) of antigen paper. (4) Prepare a suspension of human cells 1 drop of blood to 4 c.c. NaCl 0.95 per cent. It is best to prepare about 60 c.c. of NaCl solution and allow 15 drops of blood to fall from the experimenter's finger into the solution. The human cell suspension is placed over night in the ice box. Next morning the supernatant clear salt solution is pipetted off and a fresh quantity of NaCl is added (about 55 c.c.) to the cells in the beaker. Of this cell suspension add 1 c.c. to every tube in the rack. (5) Incubate for three-quarters or one hour at 38° or 39°, preferably in a large dish of warm water. Occasionally shake the tubes, to insure proper solution of the biological substance on the antigen paper. (6) Add to each tube (after incubation), front and back rows, one piece of amboceptor paper more or less, the quantity depending on the titre) and replace in the incubator, observing the result after ten minutes, and watching carefully the controls.

It will be noted in about fifteen minutes, more or less, that the rear row begins to get clear, and when complete transparency is obtained the test and control tube are to be removed to a cool place and observed. If the reaction is positive, then the front tube (test) will be opaque, in marked contrast to the control, which is transparent. For convenience of observation, I make use of a fine sealed tube (about 1 mm. in diameter) filled with black ink, which, when placed behind the control, will appear as a clear black line, whereas the positive tube will not show the black line, or it appears as a dim shadow—depending on the strength of the reaction.

It has been stated that a positive Noguchi test and a negative Wasserman is often due to the presence in the patient's serum of antisheep amboceptors. It is not necessary to perform this test with every serum as a control. Only sera giving the above results need be subjected to a verification. To demonstrate the antisheep amboceptor, place 1 c.c. of a 5 per cent. suspension of sheep cells in a test-tube, add 0.2 c.c. of patient's serum and 0.1 c.c. complement, add 3 c.c. of NaCl solution, place in incubator, and observe. If the amboceptor is present, the cells will dissolve and the mixture become transparent. The time consumed depends upon the number of amboceptor units present. I observed sera, capable of hemolyzing the cells completely in ten minutes.

Rationale of Controls. In the Wasserman and Noguchi reactions it is of vital importance to have every possible error excluded. For this purpose the controls used will answer. The substances to be controlled are the antigen, the amboceptor, and each individual serum.

The Antigen Control. This biological reagent, as is known, can per se inhibit hemolysis. To measure the degree of such interference, a tube containing a well-known normal serum (or, as I found just as serviceable, no serum at all), plus antigen, plus complement, and antisheep amboceptor plus sheep cells ought to hemolyze in about twenty to thirty minutes. No reaction is to be considered as finished before the antigen control tube is completely hemolyzed.

The Amboceptor Control. Upon the efficiency of the antisheep amboceptor depends the rapidity of hemolysis of the sheep cells. It is therefore necessary to establish the amboceptor efficiency in a separate tube containing sheep cells, plus complement, plus antisheep amboceptor. It is not essential to add normal serum. The tube containing the above ingredients is always the first to hemolyze, requiring about fifteen to twenty minutes for a complete hemolysis.

Control for Each Serum. Every serum more or less has the power to interfere with hemolysis to a slight degree. In order to control the factor of individual inhibition, every serum tested is placed in each of two tubes, the front tube contains the antigen and all other biological reagents, the rear tube receives everything but the antigen. This shows the degree of individual inhibition as compared with the tube containing the amboceptor control.

Efficiency of the Entire System. For this a well-known luetic serum is utilized. The reaction is to be positive, and hemolysis should not occur in the front tube, even if exposed to incubation temperature for hours after the controls hemolyzed.

EQUIPMENT. At least one dozen or more of Mohr's pipettes, 1 c.c., graduated into $\frac{1}{100}$. One dozen 10 c.c. pipettes, graduated into $\frac{1}{10}$. One gross of ordinary test tubes. One gross of test-tubes 1 cm. in diameter, 12 cm. high. One-quarter dozen of graduated cylinders, 50 c.c.; $\frac{1}{4}$ dozen 100 c.c. Two 50 c.c. measuring flasks with glass stoppers. A few pounds of glass tubing, 5 mm. bore, to make capillary pipettes. One-half dozen test-tube racks for Wasserman tubes; $\frac{1}{2}$ dozen test-tube racks for Noguchi tubes. A piece of rubber tubing for tourniquet. One artery clamp for above. One dozen hypodermic needles, 19 bore. One thermostat regulated at 57° and one regulated at 37°. One electric centrifuge. Labels and pencil for writing on glass. One tall glass jar for flushing through used pipettes, height to be greater than any pipette used. One dozen Petri dishes. One dozen beakers, 100 c.c. capacity. Two fine forceps, and two Hagedorn needles. One package of quantitative filter paper. One razor (for killing guinea-pigs). One 15 c.c. Luer syringe.

PREPARATION OF ANIMALS. *Antisheep Amboceptors.* Several healthy rabbits (not less than four) receive every fifth day 1, 2, 3, 4, and 6 c.c. of well-washed sheep cells. This number of rabbits is used, as one or two may die during the injection weeks. The cells are obtained from the slaughter house and immediately defibrinated with a wire defibrinator or glass beads. In order thoroughly to wash the cells a high speed centrifuge is necessary, capable of making at least 3000 revolutions to the minute. Two of the centrifuge tubes are filled with the fluid sheep blood, it being advisable, in order not to spoil the centrifuge, to have them of equal weight. The first centrifugalization brings the cells to the bottom, and the clear supernatant serum is pipetted off. The cells are now mixed with 0.95 per cent. NaCl solution and centrifugalized again, and the supernatant clear fluid is again pipetted off; this is repeated three times. The cells are now approximately serum-free. The entire quantity of cells in the tube is now brought up to its original volume with 0.95 per cent. NaCl solution, and of this, 2 c.c. is used for the first injection. With a sterile glass syringe this quantity is injected into the peritoneal cavity, having previously shaved and cleaned the puncture area. Cotton and collodion prevent wound infection. This procedure is repeated five days later with 4 c.c. of cells brought to its original volume, etc., until each animal has been injected five times. Nine days having elapsed since the fifth injection, the serum of the rabbit contains now a high lytic power against the red blood corpuscles of the sheep. The rabbit is killed and its serum used.

In the Wasserman reaction 1 c.c. of a 5 per cent. suspension of well-washed sheep red cells in 0.95 per cent. NaCl solution is the standard dose for each test. It is evident, therefore, that in order to test the power of our rabbit serum (antisheep amboceptor, as it is now called) we must use this quantity of sheep cells. Into each of six test-tubes is placed 1 c.c. of a 5 per cent. suspension of sheep cells in NaCl 0.95 per cent. These are marked from 1 to 6, and to each is added 0.1 c.c. of fresh guinea-pig serum (this is known as the complement serum and is the quantity used in the Wasserman test). We now add to test-tube (1) 1 c.c. of a 1 to 200 solution of our amboceptor. To test-tube (2) we add 1 c.c. of 1 to 400; to test-tube (3), 1 c.c. of 1 to 800; to test-tube (4), 1 c.c. of 1 to 1600; to test-tube (5), 1 c.c. of 1 to 3200. This is placed into the thermostat at 37° and the result noticed after fifteen minutes, thirty minutes, up to two hours. It will be seen that in fifteen minutes test-tube (1) is clearing up, or is clear (hemolysis); this would indicate that 1 c.c. of a 1 to 200 solution of our amboceptor is capable of destroying in fifteen minutes 1 c.c. of a 5 per cent. suspension of sheep cells. This proportion—1 to 200—is too strong, and may give negative results with some positive sera. The unit strength of the antisheep amboceptor is usually twice the quantity capable of hemolyzing the 1 c.c. of cells in two hours. If 1 to 1600 shows hemolysis after two hours and 1 to 3200 does not, then 1600 divided by 2 is the strength of the amboceptor, one unit equals 1 to 800. It is best to run two series of titration—one like the above, the second beginning with 1 to 250, 1 to 500, 1 to 1000, etc., so that a proper mean can be established and a more exact unit made. In table form the above is expressed as follows:

		Sheep cells.	Cells hemolyzed in
Tube 1 amboceptor = 1 to 200	Complement 0.1	5% 1 c.c.	15 minutes.
" 2 " = 1 to 400	" 0.1	" "	30 minutes.
" 3 " = 1 to 800	" 0.1	" "	50 minutes.
" 4 " = 1 to 1600	" 0.1	" "	1½ hours.
" 5 " = 1 to 3200	" 0.1	" "	2 hours.

Strength of 1 unit, 1 to 1600; dose for 1 test, 1 to 800. Date................

A full-grown rabbit usually furnishes from 50 to 60 c.c. of serum. This is to be kept in a glass-stoppered flask in the ice box (lower compartment). The hemolytic power does not indefinitely remain the same as in the beginning; it is, therefore, necessary to establish the titre at least once every week, and to make up the dilutions accordingly. These dilutions are to be prepared on the day of testing. The rabbit serum does not have to be inactivated to get rid of the complement in it, as the quantity of serum used is too small to influence in any way the resulting outcome of the test.

Preparation of Complement. A full-grown guinea-pig is held over a Petri dish, and after having it narcotized, the bloodvessels of the neck are severed with a razor. Suspended by the hind legs the animal is exsanguinated, and the collected blood is permitted to remain at room temperature for at least three hours. The serum collects in large drops and may be pipetted off, or the coagulum plus the serum is placed in a centrifuge tube and after five minutes centrifugalization the supernatant serum is pipetted off into a sterile test-tube; but such a serum is not as reliable as when left for three hours with its cells. About 6 c.c. of complement is obtained from one guinea-pig.

Preparation of Sheep Cells. Obtained from the slaughter house, the cells are washed three times with 0.95 per cent. NaCl solution, and 1 or 2 c.c. is mixed with 20 or 40 c.c. of salt solution, making a 5 per cent. suspension of cells.

Preparation of Antigen. The fresh liver of a luetic fœtus or the liver of any baby cadaver is chopped up very finely, and the mass is spread on a few Petri dishes and dried. The drying process is hastened by a current of air produced by an ordinary electric fan. Lately, not only baby livers, but also the livers of dogs, the hearts of guinea-pigs, and other organs were used to make antigen. The usefulness of the antigen is only established when in actual standardization it is found serviceable and works faultlessly with decidedly syphilitic and unquestionably normal sera. Consequently it makes little difference whether one uses the extract obtained from the liver of a syphilitic fœtus or from the heart of a guinea-pig, provided they are well titrated.

It is better—according to German workers—to use more than one extract, and have a series with well standardized luetic liver antigen, one with guinea-pig heart, and another with dog liver, or normal human liver. To proceed with the making of antigen the obtained dried liver is rubbed into a powder and kept in an exsiccator over $CaCl_2$ in a cool, dark place. According to Tschernogubow, such a powder is serviceable for a very long time. Of this powder, 0.5 gram is extracted at room temperature or in an ice box with 25 c.c. of 95 per cent. alcohol for twenty hours, then filtered, and the filtrate used for experiments.

For the actual Wasserman test, one part of this opalescent filtrate is diluted with five parts of 0.95 per cent. NaCl, and 0.5 to 1 c.c. used for each test-tube, the dose depending upon the established titre. The above process extracts from the liver substances soluble in alcohol, chiefly bodies of a fatty nature (lipoids). There are other means of obtaining lipoids, the above being one of the simplest, having also in view the preservation of the antigen in an active form. The liver, instead of being dried and powdered, may be directly extracted with five volumes of absolute alcohol, and the extract obtained by driving the alcohol off at a temperature not higher than 40° C. or with the electric fan. The obtained extract is much more powerful than the above, is soluble in ether, from which NaCl solutions are made for use. The titre is established carefully as follows:

Titration of Antigen. The unit dose of antigen must be of such a strength that one unit will completely inhibit hemolysis of 1 c.c. of a 5 per cent. suspension of sheep cells, with 0.2 c.c. of a known luetic serum plus 0.1 c.c. of complement; provided double this dose does not interfere with the complete hemolysis of cells using a known normal serum and complement.

TABLE OF ANTIGEN STANDARDIZATION.

Luetic Series.			*Normal Series.*	
Each tube contains syphilitic serum, 0.2; complement, 0.1; cells, 1 c.c. 5%: amboceptor, 2 units.			Each tube contains normal serum 0.2, complement, 0.1; cells, 1 c.c., 5%; amboceptor, 2 units.	
				Hemolysis.
Tube 1, Antigen,	0.025	1 hour 15 min.	Antigen, 0.025	15 minutes.
" 2, "	0.05	1 " 25 "	" 0.05	15 "
" 3, "	0.075	2 " 50 "	" 0.75	15 "
" 4, "	0.10	No hemolysis after 24 hours in incubator.	" 0.10	20 "
" 5, "	0.15		" 0.15	30 "
" 6, "	0.20		" 0.20	35 "

Dose of one unit, 0.1 c.c.

From the above facts it is evident that the dose next to the largest hemolyzing dose is the strength of one unit, or 0.1 c.c. It is also apparent that 0.2 c.c., or a double dose, will not inhibit hemolysis when used with a normal serum.

In establishing the unit dose of antigen as well as antisheep amboceptor it is of utmost importance to titrate two or three times in order to get as uniform results as possible, and only uniform work will enable one to come to a proper conclusion as to which is the necessary dose. For establishing the strength of the antigen and amboceptor, well-known fresh luetic and normal sera are to be used, as well as fresh suspension of cells and fresh complement.

Before using the standardized reagents it is advisable to perform two or three actual tests with well-known positive and negative sera. After this the substances may be considered safe for use. The above lines will give one a fair idea concerning the preparation of the biological reagents for the Wasserman reaction. For the Noguchi reaction it will be necessary, first, to acquaint the reader with the principles involved, and then the preparation of reagents will follow.

PRINCIPLES OF THE NOGUCHI TEST. Workers with the Wasserman reaction often could not explain why a positive result could not be obtained with some true luetic sera. Later it was demonstrated that this was due to the presence in the human serum of substances capable of dissolving the red blood corpuscles of the sheep; in other words, some human sera contained antisheep amboceptors. From an analysis of the workings of the Wasserman test, one could offer an objection to the statement by pointing out the fact that even in the presence of natural antisheep amboceptors, there would be no complement to bring about lysis in the hemolytic system, for the complement has been bound up in a luetic serum by the antigen. This is certainly true to some extent, but it is also true that in the presence of an excess of amboceptors, a very small quantity of unbound complement will suffice to bring about complete solution of the sheep cells used in the test. Now we very well know that in performing the Wasserman reaction we are dealing with an unknown quantity, this unknown quantity being the number of syphilitic antibody units (or some substance capable of binding complement with the antigen) present in the luetic serum. It needs no comment that the stronger and more virulent the infection, the greater the number of such bodies in the serum and naturally the firmer and more complete the binding of the complement. Sometimes the quantity of antibody is so small that a goodly portion of the complement escapes unbound and does its work by bringing about partial hemolysis; this is the condition of affairs taking place in some weak reactions. If such a serum contained antisheep amboceptors, they would have enough complement to cause hemolysis and render the result negative.

An old experiment of Ehrlich and Morgenroth proves that the greater the number of amboceptor units, the smaller the quantity of complement necessary to bring about complete hemolysis. If the patient's serum contain 10 or 20 units of antisheep amboceptor, and if only $\frac{1}{10}$ or $\frac{1}{20}$ of a unit of complement escapes deviation, this quantity will prove amply sufficient to cause hemolysis. In the Noguchi test this cannot take place, for the hemolytic system used consists of human cells plus antihuman amboceptor, and, naturally, the human organism does not contain antihuman amboceptors. Luetic sera containing antisheep amboceptors will give a negative Wasserman but a positive Noguchi test.

PREPARATION OF REAGENTS. *Antihuman Amboceptor.* Rabbits are injected with human cells the same as the sheep rabbits. After nine days the rabbits are killed, their serum collected and disposed of as follows: The fluid amboceptor loses strength on standing, so much so that it may not contain one-fourth of its original power a month after the first titration. In a dry state it can be used for a very long time without losing its strength. I prepare my antihuman amboceptor by cutting quantitative filter paper in 5 mm. squares. These squares are stuck on to pins fastened to a cardboard. With a very fine capillary pipette (as fine a one as can be made) one drop is blown on each piece of filter paper and placed in the thermostat for drying. In half an hour the papers are dry and fit for use. By this mothed each square receives exactly the same quantity of serum, and is not subject to differences in dissemination which must be considered when the serum is blown on a larger piece of filter paper and cut subsequently in 5 mm. squares. My method takes longer, but the difference is worth while, for each square holds exactly the same quantity of amboceptor. My present amboceptor is two and one-half months old and as good as new. In my work I find one piece of this amboceptor serviceable.

TITRATION OF ANTIHUMAN AMBOCEPTORS.

	Human cells 1 drop to 4 c.c. NaCl.	Complement.	Hemolysis in
Tube 1, Amboceptor, ½ piece	1 c.c.	0.05	2 hours.
" 2 " 1 "	1 c.c.	0.05	20 "
" 3 " 1½ "	1 c.c.	0.05	11 "
" 4 " 2 "	1 c.c.	0.05	8 "

As a result of five months' work with the Wasserman and Noguchi tests, I am enabled by this time to give a fair opinion as to its uses. Shortly stated, the two reactions are to my mind of the foremost importance to the clinician, and so far as accuracy is concerned, they almost occupy the first place among our means of detecting disease. The above statement is not at all to be marked as enthusiastic, for I must admit that six or eight weeks of work was spent on a very rough road to overcoming difficulties and getting acquainted with the peculiarities of the reaction, differences in the bloods submitted for tests, changes in the biological reagents, etc. My first

300 tests contained about 30 per cent. error, which by steadfast application and constant research into the causes for these mistakes, enabled me to weed them out to such an extent that now, after five months' work, I am in a position to report in 100 luetic sera, 98 to 99 positive results. In the following lines will be given the means of arriving at such a degree of precision.

First, no one who has worked with the Wasserman reaction alone has a right to claim infallibility. This is especially true for the first two months of work. Constant application and undivided attention are the prequisites for thoroughness, and only such workers are to be depended upon for their results. I believe it to be almost impossible to arrive at a high degree of technical skill without the above conditions. The handling of pipettes, even by laboratory workers, has to be improved and rendered faultlessly exact, for a drop more or less may sometimes mean a false diagnosis. As to the use of the Wasserman test alone, without the Noguchi modification, I would consider as lacking in proper controls, for, as stated before, the Noguchi modification eliminates the possibility of a negative result when antisheep amboceptors are present; and permit me to emphasize that they are present quite frequently, being the *raison d'être* of negative results in about 8 or 9 per cent. of Wasserman tests with luetic sera. Using the Noguchi test with the Wasserman places one on a much surer footing, and not only gives one better results, but also enables the worker to detect inaccuracies in the biological reagents used in the Wasserman tests.

As mentioned before, the Wasserman reaction gave a negative result in 8 or 9 per cent. of syphilitic sera. This rather undesirably high percentage of error was reduced to 1 or 1.5 per cent. when using the Noguchi and the Wasserman combined. It became apparent from the first 100 or 150 reactions that the Noguchi test, besides giving a positive reaction with all luetic sera (99 times out of 100), will sometimes give a positive reaction with a negative serum, and this to an extent of 7 per cent. As alarming as such a high degree of inaccuracy may at first sight appear, it becomes irrelevant when used with the Wasserman reaction. I have formulated a system of recording the results, which, at a glance, will give the proper interpretation of the findings in the two methods. The table is an outcome of 1400 reactions, and with it 98.2 of correct interpretations were obtained. Let me impress upon the worker the necessity for more than one test on each patient. I never render a decision after one test. Always perform two Wasserman and two Noguchi tests on different days, using the same serum. It is also to be borne in mind that a fairly marked Wasserman reaction, 99 times out of 100, means syphilis, and that a negative Noguchi the same number of times means no syphilis. The two methods are very decisive, but in opposite ways; and used together, carry with them an assurance which no amount of thoroughness and precision will replace if only one method is used. I, therefore, recommend, and am sure will be supported by other workers, that the Wasserman reaction is much less conclusive when performed without the Noguchi test, and that in the combination of the two methods we really possess a very valuable diagnostic aid.

TABLE OF INTERPRETATION OF RESULTS.

	Wasserman test first day.	Noguchi test first day.	Wasserman test second day.	Noguchi test second day.	Opinion formed.
1	S+	S+	S+	S+	Unquestionably syphilitic.
2	+	+	+	+	Unquestionably syphilitic.
3	+	—	+	—	This happens rarely, and if technique is correct, means positive. Should be done three times.
4	—	+	—	+	If antisheep amboceptors are present = positive; if no antisheep amboceptors are present = negative. Should be done three times.
5	—	W+	—	+	Suspicious. Must be decided clinically
6	—	W+	W+	W+	Positive, but weak.
7	—	S+	—	S+	Some fault with the reagents in Wasserman test, unless antisheep amboceptors are demonstrated, then positive.
8	W+	+	—	W+	= positive.
9	—	W+	—	W+	= negative, even if antisheep amboceptors are demonstrable.
10	—	+	—	—	= negative.
11	—	—	W+	+	Faulty technique. Repeat twice.
12	W+	W+	—	—	= negative. Repeat.

S+ = strongly positive.
W+ = weakly positive.
+ = ordinary reaction.
— = negative.

The above table is compiled from numerous actual observations, and is not influenced by the personal equation of the worker. To eliminate this factor, I have performed the last few hundred tests without a knowledge of the patient's condition. In many instances the test-tubes with the patient's blood were given me bearing only a number for identification, and only after rendition of the final result was the clinical diagnosis revealed to me. On one such occasion 12 sera out of 18 reacted positively, and proved to be from 11 cases of tabes and one of tricuspid stenosis. The latter subsequently reacted ten times positively. Such non-knowledge of the patient's condition is to be recommended if one wishes unbiassed results; and statistics obtained by such means are far more valuable than those obtained by giving the laboratory worker the diagnosis with the blood. The clinical findings belong to the doctor, and ought to be offered in exchange for the laboratory report, and not sooner.

This work comprises results from 1390 analyses. The first 300 are unreliable on account of lack of technique. The remaining 1090 carry with them a high degree of precision. Analyzing the sensitiveness of each method independently, I find that out of 503 sera examined by the Noguchi method, 43 were contrary to the diagnosis of expert clinicians. Of these 43 probably incorrect results, 35 were interpreted as positive and 8 as negative. The Wasserman reaction behaved as follows: Out of 487 sera, 37 were wrong. Of these 37, 30 were negative and 7 positive. Using the combined method of reading results—having performed two Wasserman and two Noguchi tests and then rendering a report—the results were as follows: Out of 487 reports, 9 were contrary to the clinical picture; of these, 5 were negative and 4 positive, making it less than 2 per cent. error.

Before giving my conclusions, I would like to offer a few explanations of the peculiarities and at times perplexing results obtained while working with complement deviation. On more than one occasion the presence of antisheep amboceptors has been blamed for a negative Wasserman reaction. This is true only to a certain extent, for we know that in the presence of a sufficient quantity of syphilitic antibody, a perfect and complete deviation of complement will take place with a well-standardized antigen. The antisheep amboceptor comes into prominence only when there is a paucity in luetic antibody, and then only will render the Wasserman reaction useless.

Some guinea-pigs furnish a serum which is very poor in complement, rendering the amboceptor powerless or too weak to hemolyze the given quantity of cells; in such a case the outcome is a weak positive reaction in a negative serum.

The sheep cells must also be considered, as they, too, have varying powers of resistance to the amboceptor, and will sometimes remain intact for hours. This is one of the reasons why some controls and tests react very sluggishly. Another reason for a prolonged resistance to the amboceptor is the presence in the patient's serum of anticomplemental substances which hinder or render inert the completing function of the complement. In such a case the addition of another half dose of guinea-pig serum will bring out clearly the nature of the serum, and also decide its luetic or negative qualities.

Although the above deviations cause delay, puzzle and discourage the beginner, by far the greatest source of error lies in the worker himself, especially when a drop more or less is considered as insignificant, or any pipette as clean enough. Thorough cleanliness, using sterile dry pipettes and containers, as well as faultless pipettation, are conditions *sine qua non* in the performance of the Wasserman and Noguchi tests; they are only acquired after weeks of constant work and application. I do not wish by these statements to discourage anybody from working with the methods, but I would advise the worker not to place himself on record before every detail has been mastered and he is able to answer why and wherefore certain tests were negative on one occasion and again positive on another day, and which of the two is correct. The laboratory worker, being responsible to the clinician for his statements, ought to be in a position to help him considerably, but only when his work has been carried out very carefully, unbiassed by personal opinion, and submitting the result of a delicate test as read from the test-tube. I do not believe we are as yet in a position to say that a serum preventing the hemolysis of sheep cells in the Wasserman test, or human cells in the Noguchi modification, means the presence of syphilis. Leprosy gives it, trypanosomiasis is reported to give the reaction, and undoubtedly, there are other conditions which give rise to the formation of substances in a patient's serum which, when brought in contact with antigen (lipoid bodies), are capable of inhibiting, destroying, or deviating the completing action of the complement, so that hemolysis will not take place when a specific hemolysin and cells are placed in such a mixture. I had occasion to analyze two cases of scleroderma, and found in both the reaction positive. Whatever the process may be, the explanation of its modus operandi is still a theory made to fit the well-known exposition of Ehrlich's concerned in immune processes, which in itself is only an ingenious theory. The practical man wants a reliable report on the blood submitted for analysis. In my case, only yes or no was accepted. I was confronted with questions as to the significance of a weak reaction, an ordinary and a strong reaction. From the above remarks it is apparent that one is hardly justified to state positively that even a strong reaction signifies, without the least doubt, syphilis. In the vast majority of bloods syphilis is most likely the cause for a strong reaction, but it need not at the same time be the only factor responsible for its appearance.

A strong reaction naturally carries with it a greater degree of assurance in rendering a decision than a weak reaction. This is especially true of the Wasserman test, for at no time was a strong reaction obtained with this method unless the blood came from a patient giving clinically or anamnestically signs of syphilis. A strong positive reaction with the Noguchi test is much less significant, at least in my experience. I obtained positive results with the Noguchi method when, from the physical examination and the history, syphilis could be excluded to the satisfaction of the clinician. On the other hand, a distinctly negative result, using the Noguchi method, is very valuable, and in the majority of instances comes from an individual free from a syphilitic taint, as corroborated subsequently by a careful examination.

In the above work no deviation from the accepted technique was resorted to. In the Noguchi tests personal communication with the originator of the method greatly facilitated the progress of my work by rendering clear some obscure phenomena and furnishing a re-

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liable antigen. The amboceptor I prepare myself by a slightly different method, the aim being greater uniformity in the 5 mm. squares of paper used in the tests. In every other detail the technique is exactly as advocated by Dr. Noguchi, using the same reagents, and working with the same controls.

CONCLUSIONS. 1. Every beginner, for the first month or two, will obtain unsatisfactory results from lack of proper technique.

2. Having mastered the technical difficulties, always perform another test for verification.

3. Acquire the habit of working with two methods, the Wasserman and Noguchi.

4. Every week or ten days, spend a day in standardizing reagents.

5. With proper technique and well balanced reagents it is possible to report correctly in 98 per cent. of cases.

6. Questionable reactions are not to be used for diagnosis, and if a given serum does not react strongly after a number of repetitions of the tests, the diagnosis is to be left to the clinician.

7. I find that the Wasserman reaction gives negative results in about 7 per cent. of positive sera, treatment periods excluded.

8. The Noguchi method gives 8 per cent. of positive results in clinically well-established non-syphilitic individuals. The positive reactions in these cases are ordinary reactions, and cannot be said to be remarkably strong.

9. Exceptionally strong reactions are obtained in untreated cases of general paresis with both tests, as well as in primary sores four weeks after infection.

10. With time, the antibody content of the serum diminishes. The antibody content also diminishes during inactivation to about one-third. (Personal communication by Dr. Noguchi.)

11. The table of interpretation of results was of great help in rendering many correct reports, and served to some extent as a guide in the disposal of the serum submitted for testing.

12. The compilation of this table took place after analyzing the results from 1390 reactions.

13. I do not feel justified to place myself on record concerning the specific or non-specific nature of certain diseases. However, the majority of sera from tabetics (78 per cent.) and most sera from general paretics (not treated recently) deviate complement, and the latter most strongly. In diabetics a positive result could rarely be had. The few cases of aneurysm and aortic lesions as well as one case of tricuspid stenosis, all gave a positive result. Out of 84 locomotor ataxia cases, 66 gave a positive Wasserman and 70 a positive Noguchi reaction. The findings in clinical conditions will be the subject of another communication.

14. I am not at present in a position to state the influence of treatment on the reaction, and to what extent or manner my method of interpretation will be influenced.

I wish to thank all gentlemen who were helpful in furnishing material for work, my gratitude being due to Dr. I. Abrahamson for initiating this work in the Montefiore Home Laboratory, to Dr. Noguchi for his generous help in elucidating complex phenomena and furnishing some reagents, and to Drs. H. Brooks and S. Wachsmann for encouraging and facilitating the research.

A STUDY OF THE ALLEGED PRESENCE OF TUBERCLE BACILLI IN THE CIRCULATING BLOOD.

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Twelve months have now elapsed since Rosenberger[1] read his initial paper before the Pathological Society of Philadelphia, upon the presence of tubercle bacilli in the blood. This paper gave rise to considerable enthusiasm in local medical circles and no little interest to the medical world at large, because he asserted that it was easily possible to demonstrate the presence of tubercle bacilli in the blood of every case of tuberculosis, by means of a technique so simple that it became a matter of surprise that previous efforts, along this same line, by many competent investigators, should not long ago have been rewarded with equal success. Many accepted his findings quite readily, while others listened to them with a good deal of reservation.

Rosenberger's original studies comprised 50 cases divided as follows: 2 cases of fibroid tuberculosis; 1 case of pneumothorax; fifteen cases of incipient tuberculosis; 23 cases of advanced tuberculosis, and 3 cases of the laryngeal form of the disease. He has since augmented this number, so that at the present time they embrace over 300 observations, including cases of lupus. In all of these cases he has met with the same success, finding the bacillus in the blood in every instance.

This contribution was immensely important, because, if correct, some of our preconceived ideas concerning tuberculosis must be modified, and because it would afford the clinician a simple and almost infallible means of diagnosticating the malady in its earliest incipiency, a matter at the present time perplexing and difficult, if not impossible. In lieu of all this it is astonishing to find that, with the exception of two brief contributions on the subject, Rosenberger's observations have not been confirmed.

In reviewing the literature in the chronological order in which

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[1] Amer. Jour. Med. Sci., 1909, cxxxvii.